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Trophic factors for Parkinson's disease: Where are we and where do we go from here?

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ABSTRACT

Perhaps the most important unmet clinical need in Parkinson's disease (PD) is the development of a therapy that can slow or halt disease progression. Extensive pre-clinical research has provided evidence for the neurorestorative properties of several growth factors, yet only a few have been evaluated in clinical studies. Attempts to achieve neuroprotection by addressing cell-autonomous mechanisms and targeting dopaminergic neurons have been disappointing. Four different trophic factors have so far entered clinical trials in PD: glial cell line-derived growth factor, its close structural and functional analog neurturin, platelet-derived growth factor and cerebral dopaminergic neurotrophic factor. This article reviews the pre-clinical evidence for the neuroprotective and neurorestorative actions of these growth factors and discusses limitations of pre-clinical models, which may hamper successful translation to the clinic. We summarize the previous and ongoing clinical trials using growth factors in PD and emphasize the caveats in clinical trial design that may prevent the further development and registration of potentially neuroprotective and neurorestorative treatments for individuals suffering from PD.

Abbreviations: adeno-associated viral (AAV); adenoviral (AdV); cerebral dopaminergic neurotrophic factor (CDNF); convection-enhanced delivery (CED); dopaminergic (DA); endoplasmic reticulum (ER); GDNF family receptor (GDF); glial cell line-derived neurotrophic factor (GDNF); intracerebroventricular (ICV); lentiviral (LV); mesencephalic astrocyte-derived neurotrophic factor (MANF); neurotrophic factor (NTF); Parkinson's disease (PD); platelet-derived growth factor (PDGF-BB); positron emission tomography (PET); substantia nigra (SN); tyrosine hydroxylase (TH).

1. Trophic factors in pre-clinical models of Parkinson's disease

Neurotrophic factors (NTFs) are endogenous secreted proteins, which are necessary for the correct development, differentiation and maintenance of neurons. Individual neuronal populations require specific NTFs in a precisely-regulated spatial and temporal manner during their embryonic development and for their ongoing support throughout life. From the point of view of Parkinson's disease (PD), NTFs that act specifically on dopaminergic (DA) neurons are of interest, as there is potential to harness their survival-promoting properties to protect and repair the degenerating nigrostriatal pathway. Most NTFs act on specific transmembrane receptor complexes, activating intracellular survival and differentiation pathways. Several dopaminergic NTFs have been tested in pre-clinical *in vivo* models of PD and been found to have potent neuroprotective, neurorestorative and functional effects.

GDNF

Glial cell line-derived neurotrophic factor (GDNF) was the first NTF to show potential for PD therapy, following a report of its survival-promoting actions on cultured DA neurons (Lin *et al.*, 1993). Intracerebral application of recombinant GDNF protein has been consistently shown to confer significant neuroprotection in *in vivo* animal models of PD. The most commonly-used model is 6-hydoxydopamine (6-OHDA) lesion of the adult rat nigrostriatal pathway. Using this model, many groups have shown that stereotaxic injection of GDNF to the striatum, either before or after the lesion, protects against 6-OHDA-induced neuronal death and the resulting motor dysfunction (for reviews see (Bjorklund *et al.*, 2000; Sullivan & Toulouse, 2011; Kordower & Bjorklund, 2013; Torres *et al.*, 2017). Although many of these studies administered GDNF prior to, or at the same time as, the lesion, several others have investigated effects of delayed GDNF treatment and have reported neurorestorative effects on motor symptoms and nigrostriatal integrity (Winkler *et al.*, 1996; Rosenblad *et al.*, 1998; Aoi *et al.*, 2000). Delayed administration of trophic factors, at a stage when the nigrostriatal

pathway has already degenerated significantly, is obviously more relevant to the clinical scenario. In another rodent model, l-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, GDNF administration prior to or following MPTP has also been found to exert neuroprotective or restorative effects (Tomac *et al.*, 1995; Date *et al.*, 1998). Restorative effects in MPTP-treated monkeys have also been reported; GDNF infusion at several weeks or months after establishment of MPTP lesions induced significant neurorestorative effects and reduction in parkinsonian symptoms, at a stage when the nigrostriatal pathway had already undergone significant degeneration (Gash *et al.*, 1996; Iravani *et al.*, 2001; Grondin *et al.*, 2002). These positive studies in animal models led to the initiation of clinical trials of intracerebral infusion of GDNF protein in PD patients.

Since GDNF is an endogenous protein that is rapidly metabolised in the brain, gene therapy strategies were developed with the aim to allow more targeted and sustained delivery to the nigrostriatal system. In pre-clinical studies, overexpression of GDNF has been achieved by the use of adenoviral (AdV), lentiviral (LV) and adeno-associated viral (AAV) vectors (for reviews see Bjorklund *et al.*, 2000; Domanskyi *et al.*, 2015; Kelly *et al.*, 2015). A large number of studies have reported significant neuroprotective effects and improvements in motor symptoms following GDNF delivery by AdV vector to the substantia nigra (SN) or striatum of 6-OHDA lesioned rats or MPTP-treated mice. LV vectors have also been used to deliver GDNF in animal models, conferring neuroprotective and restorative effects on the nigrostriatal pathway of 6-OHDA-lesioned rodents and MPTP-treated primates, with effects lasting for several months (Bjorklund *et al.*, 2000). AAV vectors are considered to be safer than either AdV or LV vectors since they are not linked to any human disease (see Colella *et al.*, 2018). The use of AAV vectors has proven successful for targeted and sustained GDNF delivery in 6-OHDA and MPTP rodent and primate models of PD, achieving potent neurorestorative and functional effects (Mandel *et al.*, 1997; Kirik *et al.*, 2000; Eslamboli *et al.*, 2003).

In contrast to the positive outcomes in 6-OHDA and MPTP models, GDNF delivery by either AAV or LV vectors was not effective in the AAV-α-synuclein model of PD (Decressac *et al.*, 2011). This newer model involves AAV-mediated overexpression of α-synuclein in the adult rat SN, which results in delayed and progressive degeneration of the nigrostriatal pathway (for reviews see (Ulusoy *et al.*, 2010; Lindgren *et al.*, 2012; Volpicelli-Daley *et al.*, 2016). It has an advantage over previous models since it mimics some of the pathology of the human disease, including the accumulation of α-synuclein inclusions, and displays a more protracted degeneration than occurs in other animal models. The failure of GDNF to exert neuroprotective effects in AAV-α-synuclein-treated rats (Decressac et al, 2011), and in a similar rat model involving LV-mediated overexpression of the A30P mutant human α-synuclein (Lo Bianco *et al.*, 2004), is thought to be due to α-synuclein-induced downregulation of the expression of Nurr1, and its downstream target, the GDNF receptor Ret (Decressac *et al.*, 2012).

Animal models have been useful for the optimisation of technical aspects of the protocols used to administer NTFs to patients. The development by the Bankiewicz group of a convection-enhanced delivery (CED) system to deliver AAV2-GDNF safely and effectively in rodent and primate models, including aged monkeys (Johnston *et al.*, 2009; Su *et al.*, 2009) led to the subsequent clinical application of CED-mediated AAV2-GDNF in PD patients. This group has also explored the use of gadoteridol (Gd)-enhanced magnetic resonance imaging-guided CED of AAV2-GDNF in rodents and monkeys (Su *et al.*, 2010) (Gimenez *et al.*, 2011; Richardson *et al.*, 2011), as well as optimising surgical cannula design and use (Sanftner *et al.*, 2005; Yin *et al.*, 2010a; Yin *et al.*, 2010b).

Neurturin

Neurturin is a member of the transforming growth factor β protein family and is a close structural and functional analog of GDNF (Kotzbauer *et al.*, 1996). While GDNF exerts its action by binding to GDNF family receptor (GDF) α-1 and the transmembrane receptor

tyrosine kinase, Ret, neurturin acts through Ret and GDFα-2. Neurturin was found to confer neuroprotective and functional effects on the nigrostriatal pathway in the 6-OHDA rat model, in a similar manner as GDNF (Horger *et al.*, 1998; Rosenblad *et al.*, 1999; Oiwa *et al.*, 2002; for reviews see Kelly *et al.*, 2015; Olanow *et al.*, 2015). Pre-treatment with neurturin was found to protect the nigrostriatal dopamine neurons from MPTP-induced damage in Rhesus monkeys (Li *et al.*, 2003). Of higher relevance to the clinical situation, restorative effects have been reported following neurturin delivery to the already-lesioned nigrostriatal system in 6-OHDA-treated rats (Oiwa *et al.*, 2002) and in MPTP-treated monkeys (Grondin *et al.*, 2008).

Recently it has been reported that overexpression of neurturin in the adult rat SN can protect against 6-OHDA-induced loss of DA neurons and motor function, as well as stimulating DA neurite outgrowth and increasing striatal dendritic spine density (Reyes-Corona *et al.*, 2017). However, in both rats and monkeys, the protective effects of neurturin are slightly lower than those of GDNF (for review see (Kelly *et al.*, 2015), possibly reflecting lower solubility and diffusion properties of neurturin. With the aim of circumventing the issues of poor solubility and short half-life of the recombinant protein, gene therapy approaches to introduce neurturin to the brain were designed. Intracerebral injection of LV-neurturin (Fjord-Larsen *et al.*, 2005) or AAV2-NTRN (Bartus *et al.*, 2011a; Gasmi *et al.*, 2007; Bartus *et al.*, 2011a; Herzog *et al.*, 2013) was successfully tested in 6-OHDA-lesioned rats. Subsequent studies in MPTP-lesioned monkeys (Kordower *et al.*, 2006; Herzog *et al.*, 2008; Bartus *et al.*, 2011b) and aged non-parkinsonian monkeys (Herzog *et al.*, 2007; Bartus *et al.*, 2011b) showed safety and efficacy, and led to AAV2-neurturin, under the name CERE-120, entering clinical trials in PD patients (for reviews, see Bartus *et al.*, 2013a; Kelly *et al.*, 2015).

PDGF-BB

Platelet-derived growth factors (PDGF)s are endogenous growth factors that occur in several different isoforms. The PDGF-B gene product forms the biologically active PDGF-BB dimer, that binds to PDGFRα and β, whereby it has the highest affinity for PDGFR β. Pericytes highly express the receptors for PDGF-BB, and PDGF-BB signaling is important for pericyte recruitment to the blood vessels, proliferation and migration. However, PDGF–BB also seems to play a role in the nigrostriatal system. Lesions in this system induce an elevated PDGF-B synthesis, probably due to an endogenous neuroprotective response (Nikkhah *et al.*, 1993; Funa *et al.*, 1996). Studies using pre-clinical toxin-induced PD models (partial 6-OHDA model in rats, MPTP model in mice and in non-human primates) provided compelling evidence that intracerebroventricular (ICV) infusion of PDGF-BB for two weeks restored not only DA neurotransmission, but also provided functional recovery. In PD models, injection of PDGF-BB in lesioned animals results in increased periventricular cell proliferation, increased number of nigral tyrosine hydroxylase-positive (TH) neurons, partial restoration of DA transporter (DAT) levels and normalization of behavioral deficits in rodents (Zachrisson *et al.*, 2011; Padel *et al.*, 2016) and in a nonhuman primate model (Paul *et al.*, 2013).

Surprisingly, the mechanism leading to this compelling effect is not known. An acute neuroprotective effect is unlikely because PDGF-BB was injected 5 weeks after the PD lesion in the rodent models. The effect is abolished by inhibition of cell proliferation with a mitosis inhibitor, which suggests that it is mediated via dividing cells (Zachrisson *et al.*, 2011). However, no newborn DA neurons have been found after PDGF-BB treatment. Thus, the yet unknown “dividing cell type” most likely secretes molecules that in turn have a protective and restorative effect on the remaining DA system. It has been hypothesized that PDGF-BB stimulates periventricular cell proliferation which in turn elicits direct or indirect effects on the DA cells (Zachrisson *et al.*, 2011). Candidate cell types responding to PDGF-

BB should express the receptors PDGFRα and/or β if the effect is a receptor-mediated effect. PDGFs are known to stimulate proliferation of pericytes, perivascular cells that highly express PDGFRβ.

Interestingly, a 6-OHDA lesion in mice has been associated with a pathological activation of pericytes that was normalized upon ICV administration of PDGF-BB and, as previously reported, led to nigrostriatal restoration and behavioral recovery (Padel *et al.*, 2016). PDGF-BB is known to be secreted by endothelial cells and binds the PDGFRβ on pericytes to recruit these cells to the vessel wall and to stabilize blood vessels (Winkler *et al.*, 2010; Sweeney *et al.*, 2016). Deregulated PDGF-BB/PDGFRβ signaling is one of the few pathways discovered so far to cause blood-brain barrier defects that result in secondary neurological dysfunction. The importance of this PDGF-BB/PDGFRβ pericyte signaling is underscored by findings in mice with partially disrupted PDGF-BB/PDGFRβ signaling that display an age-dependent increase in BBB breakdown and a neurodegenerative-like phenotype with progressive neuronal loss (Bell *et al.*, 2010). Thus, PDGF-BB may exert its effect on the DA system via normalization of aberrant vascular changes. In addition, PDGF-BB/PDGFRβ -signaling in pericytes has been shown to lead to secretion of different growth factors and cytokines as well as microvesicles containing growth factors implicated in neuroprotection and neurorestoration (Gaceb *et al.*, 2018a; Gaceb *et al.*, 2018b). Therefore, several non-cell autonomous mechanisms may converge to the regenerative effect seen in PD models. In addition, the vascular effects seen after ICV administration of PDGF-BB in PD (Padel *et al.*, 2016) point to new avenues for therapeutic approaches targeting the neurovascular unit.

CDNF

Cerebral dopaminergic neurotrophic factor (CDNF) and its homologue mesencephalic astrocyte-derived neurotrophic factor (MANF) belong to a family of evolutionary-conserved factors that are located and secreted in the endoplasmic reticulum (ER). CDNF has a unique

structure and dual mode of action that differs from other known NTFs (Lindholm *et al.*, 2007). CDNF has been shown to protect cells from ER stress, regulate the unfolded protein response to cellular stress, and to dissolve intracellular α-synuclein aggregates. It acts via yet to be identified plasma membrane receptors when it is secreted into the extracellular space. The therapeutic potential of CDNF has been demonstrated in both the rat 6-OHDA model and the mouse MPTP model (Voutilainen *et al.*, 2011; Airavaara *et al.*, 2012). Intrastriatal injection of AAV-CDNF has been shown to protect and recover 6-OHDA-induced behavioural deficits and to induce significant restoration of nigral DA neurons and their striatal fibres (Back *et al.*, 2013; Ren *et al.*, 2013). Following the positive outcomes in the studies on rodent models, a safety and efficacy study using chronic intrastriatal infusion of CDNF protein in 6-OHDA-lesioned marmosets (Garea-Rodriguez *et al.*, 2016) paved the way for clinical trials with this NTF.

Combined application of LV-CDNF and LV-MANF into the SN has synergistic protective effects on DA neuronal survival and on motor behaviour in 6-OHDA-lesioned rats (Cordero-Llana *et al.*, 2015). A recent study reported additive effects of GDNF and CDNF proteins in 6-OHDA-lesioned rats (Voutilainen *et al.*, 2017), probably reflecting the different mechanisms of actions of these two NTFs. It is noteworthy that this study applied the factors four weeks after the 6-OHDA lesion, showing their therapeutic potential in the already-degenerated nigrostriatal pathway, which closer resembles the clinical situation than pre-treatment with trophic factors. The approach taken in both the Cordero-Llano and Voutilainen studies, of applying a combination of two NTFs, may be worth exploring in the planning of future clinical trials. In particular, treatment with two factors that act via different receptors and intracellular signaling pathways may lead to the rescue of a larger number of degenerating DA neurons.

2. Limitations of pre-clinical models for testing trophic factors for PD

Ideally, any potential therapy for PD should be tested in animal models that best recapitulate the features of the human disease. One of the important characteristics of PD is the fact that it is a progressive disease, which is difficult to model pre-clinically. Although the use of the acute 6-OHDA median forebrain bundle lesion has largely been superseded by the intrastriatal ('partial lesion') model, which induces DA neuronal degeneration over about ten days rather than a few hours, this is still not ideal for modelling the human disease, which progresses over decades. Neither the 6-OHDA or MPTP rodent models display the Lewy body accumulation that is the primary pathological hallmark of human PD. Thus the development of α-synuclein rodent models is an important advance and has contributed much to the study of disease mechanisms and to testing of potential therapies. However, it is important to note that there is much variability in the pathological and functional features of the AAV-α-synuclein model. Individual studies have reported various extents of DA neuronal loss and of motor impairment. This likely reflects variation in the experimental methods used to create the model, for example, different serotypes of the capsid protein, or varying viral titers and doses (reviewed by Volpicelli-Daley et al., 2016). Further variability lies in the fact that some groups use overexpression of the wild-type α-synuclein protein, while others use mutated forms.

The failure of GDNF application in the AAV-α-synuclein and LV-A30P-α-synuclein rat models (Decressac *et al.*, 2011; Lo Bianco *et al.*, 2004), compared to the encouraging data from the neurotoxin-based models, highlighted the variability in pre-clinical PD models. The downregulation of Ret receptor expression by α-synuclein was thought to be the reason for the lack of neuroprotective action of GDNF, which relies on this receptor. However, another group has urged caution in the translation of these data to human PD. Using micorarray analysis, this group found no alterations in the expression of Ret or Nurr1, or indeed any

correlation between the synuclein and Nurr1 genes, in human PD samples (Su et al 2017). This study also reported no downregulation of Ret in transgenic α-synuclein mice.

A critical issue for consideration of NTFs in clinical trials is the disease stage at the point of intervention. The majority of the pre-clinical studies have administered NTFs at time of, or shortly after, the lesion. This is not a good model of advanced PD, which is the stage of most of the participants in the clinical trials to date. The use of progressive models such as the AAV-α-synuclein rat may help to circumvent this issue. Although animal models, particularly primates, have proven useful for testing some of the technical aspects of intracerebral delivery, such as CED, image-guided placement and refinements of cannula design, there are of course limitations to their ability to mimic the anatomical features of the human brain. Another important point to consider in pre-clinical modeling of PD is the increasing emphasis on the multi-system nature of this disorder. The traditional animal models exclusively target the nigrostriatal pathway, by using the selective DA neurotoxins 6-OHDA and MPTP, resulting in good models of motor dysfunction caused by the loss of these neurons, but not wholly taking into consideration the many other systems affected in human PD. There is increasing realisation that the numerous non-motor symptoms, such as autonomic, gastrointestinal, sleep and cognitive problems, have a large impact on the quality of life of PD patients. These additional symptoms should be considered when designing treatment regimens for people with PD. Some studies have characterised short-term memory deficits and depressive-like behaviours in rodents with bilateral 6-OHDA or MPTP lesions (Ferro *et al.*, 2005; Santiago *et al.*, 2010; Matheus *et al.*, 2016). However, no studies published to date have investigated the effects of trophic factors in these bilateral models of non-motor symptoms of PD. Studies by Prediger and colleagues have characterised the intranasal route of delivering MPTP to both rats and mice, which causes loss of neurons in olfactory bulb and SN (Prediger *et al.*, 2011). These rodents exhibit olfactory, cognitive and emotional defects as well as motor symptoms (Prediger *et al.*, 2009; Prediger *et al.*, 2010), making this a valuable model in which to test trophic factors and other novel therapeutics. Another group

used 6-OHDA lesions of both nigrostriatal and mesolimbic dopamine pathways in rats and reported depressive-like symptoms but no deficits in anxiety-like or cognitive behaviours (Carvalho *et al.*, 2013). There has been some progress in modeling of cognitive symptoms in the AAV-α-synuclein rat model (Campos *et al.*, 2013; Crowley *et al.*, 2018), but further pre-clinical work is needed to address the complexity of systems that are affected in PD and how therapies may impact upon the resulting symptoms.

There is currently no pre-clinical model of PD that fully demonstrates all of the features, construct validity (is based upon recapitulating disease mechanisms and/or neuropathological features), face validity (replicates the disease symptoms and features), as well as predictive validity (reliably predicts patients' responses to treatments). In terms of face validity, the 6-OHDA and MPTP rodent models do recapitulate most of the cardinal motor symptoms of human PD, as well as the death of nigrostriatal DA neurons. However, they do not exhibit many other motor symptoms (such as rigidity, tremor or balance disturbances) that are characteristic of the human disease, nor do they exhibit many of the non-motor symptoms. The genetic models exhibit construct validity, since they are created based on a mechanism that is relevant to the disease. For example, models involving a-synuclein or LRRK2 mutations, and PINK-1, Parkin or DJ-1 knockout, are based on knowledge that mutations in these genes causes PD in humans. However, for the most part, these genetic models have not been shown to produce neurodegeneration and/or motor symptoms - and so do not fully recapitulate the disease. The neurotoxin-based rodent models do not exhibit good construct validity since they do not recapitulate the primary neuropathological feature of PD, that is, alpha-synuclein accumulation. In terms of predictive validity, although most of the models show a response to L-DOPA and other therapies routinely used in PD patients, they do not all allow measurement of all symptoms of human PD. For example, it is very difficult to measure fine motor control in rodents. The MPTP primate model allows this type of measurement and therefore has higher predictive validity for testing drugs aimed at improving this aspect of the disease symptoms. Perhaps any

potential treatments should be tested in both neurotoxin and α-synuclein models, at least until an ideal animal model emerges. Care should be taken when translating data from studies involving trophic factors pre- or co-administered with a neurotoxin; studies that administer factors to animals with already-established lesions better reproduce the clinical situation, where patients present with already significant nigrostriatal damage. It will also be important to evaluate the efficacy of NTFs in animal models which also incorporate standard anti-parkinsons medications. For example, one study on MPTP-treated marmosets found that intraventricular administration of GDNF reduced the severity of L-DOPA-induced dyskinesias, as well as improving motor function and restoring the integrity of the nigrostriatal dopaminergic system (Iravani et al., 2001). Studies on animal models will also be crucial for investigations of disease mechanisms, to enable optimisation of NTF therapy. Furthermore, pre-clinical work on the mechanism(s) of action of known and novel NTFs will be critical, to allow the identification of 'druggable' targets and the development of therapies based on small molecules which can activate downstream signaling pathways. For example, i.p. administration of deoxygedunin, a small molecule TrkB (brain-derived neurotrophic factor receptor) agonist, was reported to exert neuroprotective and behavioural effects in both MPTP and 6-OHDA rodent models of PD (Nie *et al.*, 2015). Important work by Burke and colleagues has shown restorative effects on the nigrostriatal pathway in the 6-OHDA rat PD model, by upregulation of the activity of Akt kinase (Padmanabhan & Burke, 2018), a critical downstream player in the signalling pathways used by several neurotrophic factors. In vitro studies have shown that synthetic forms of microRNA(miR)-182-5p and miR-183-5p, downstream mediators of GDNF's signalling pathways, can exert neuroprotective and trophic effects on midbrain DA neurons (Roser *et al.*, 2018). Another study found that inhibition of miR-181a, a downstream negative regulator of BMP/GDF neurotrophin signalling, can promote DA neuronal growth *in vitro* (Hegarty et al, 2018). These studies highlight the therapeutic potential of targeting small molecules, such as miRs, as activators of neurotrophin signalling, in the development of drugs that can be administered

systemically. The ultimate goal is to design a safe and efficacious therapy which can be given to patients without the need for invasive neurosurgery.

3. Trophic factors that entered clinical trials for PD

As described above, several NTFs have shown convincing pre-clinical evidence for a restorative effect in PD and, based on those findings, entered clinical trials (see Table 1).

GDNF

GDNF has so far been studied in seven clinical trials, two of which are still ongoing or under evaluation (see Table 1). Initially administered to the brain via ICV infusion, GDNF had a lack of efficacy despite being tested in a multicenter, randomized, double-blind placebo-controlled study design in 50 patients, exploring seven different dosages of GDNF. Injections were given monthly over 8 months followed by an open-labeled extension of 20 months. Instead, side effects such as nausea, weight loss and asymptomatic hyponatremia were reported (Nutt *et al.*, 2003). The lack of a clinical effect in this trial was attributed to the fact that GDNF when delivered into the ventricles most likely did not reach the target tissue it was intended for, the putamen and SN. Subsequently, intraparenchymal delivery of GDNF directly into the putamen was explored instead. In an open-label study of continuous intraputamenal GDNF infusion in five patients (one unilaterally and four bilaterally), clinical improvement was not only evident within three months of the treatment, but the clinical effect was sustained and progressive. By 24 months, patients demonstrated a 57 and 63% improvement in their off-medication motor and activities of daily living UPDRS subscores, respectively, and a clear benefit on dyskinesias. The benefit was associated with a significant increase in putamenal ^{18}F-dopa uptake on positron emission tomography (PET) (Gill *et al.*, 2003). One patient came to autopsy after 43 months of unilateral infusion and

post mortem analysis showed increased TH-immunopositive nerve fibres in the infused putamen (Love *et al.*, 2005). A second open-labeled trial in 10 patients using unilateral intraputamenal GDNF infusions similarly demonstrated a greater than 30% benefit in both on- and off-medication scores at 24 weeks (Slevin *et al.*, 2005), that reached 45% at 1 year (Slevin *et al.*, 2007). Effects were maintained up to 9 months after cessation of the treatment (Slevin *et al.*, 2007).

Due to the promising results of these open-labeled trials using intraputamenal infusion, hopes were fueled to have found a factor that exerts clinical benefit in PD. The two open-labeled trials were followed by a phase II randomized-controlled trial including a placebo-group (N=34, 1:1 randomization). Here, GDNF was administered continuously bilaterally into the posterior dorsal putamen (corresponding to its sensorimotor part) using a chronic infusion pump and patients were evaluated at 6 months. Much to the disappointment of patients and the scientific community who were eagerly awaiting the results, the relative difference between the treated and the placebo group did not reach statistical significance. In addition, a small number of patients (n=3) developed neutralizing antibodies (Lang *et al.*, 2006). These findings, together with reported Purkinje cell loss in a few GDNF-treated monkeys (Hovland *et al.*, 2007), led to study withdrawal by Amgen. This decision was met with strong feelings by patients, especially since individual patients who had participated in the study witnessed clinical improvement, similar to published single case reports describing clinical improvement several years after cessation of the drug treatment (Love *et al.*, 2005; Patel *et al.*, 2013).

After several years and unsuccessful clinical trials using neurturin (see below), persistence prevailed. Two clinical trials are currently ongoing focusing on increasing the putamenal coverage of the infused GDNF. A new single-center phase II randomized placebo-controlled study was initiated including 41 PD patients in Bristol (www.parkinsons.org.uk). The Bristol colleagues used a new infusion protocol for GDNF protein delivery, involving a CED system. This clinical study is now concluded. First data were announced reporting that there was no

significant difference in the UPDRS motor score compared to the placebo group at the first endpoint of 9 months (http://medgenesis.com/news.htm). The study had an open-labeled extension for another 9 months where all participants received active treatment and these results are currently eagerly awaited.

Another way to enhance distribution is delivery of GDNF using viral vectors that may circumvent issues of limited diffusion and pulsatile stimulation. AAV, specifically the AAV2 subtype, is currently the vector of choice for clinical trials in PD. This virus does not induce an inflammatory response, is relatively safe and enables long-term expression of the transgene. An ongoing phase I single-center open-label dose escalation study is currently investigating the safety and tolerability of AAV2-GDNF in 25 PD patients at the National Institute of Neurological Disorders and Stroke. Four escalating dose levels are being evaluated. The gene is bilaterally delivered to the putamen using a CED system (ClinicalTrials.gov Identifier: NCT01621581), but results are not to be expected for another few years.

Neurturin

The withdrawal of GDNF by Amgen renewed the interest in the closely related neurturin, which had shown promise in pre-clinical studies. This led to a Ceregene-initiated open-labeled clinical trial using AAV2-mediated gene transfer of neurturin delivered into the striatum (Marks *et al.*, 2008). This safety study showed promising results, but again disappointed when a subsequent randomized placebo-controlled trial using AAV2-NTN showed only modest benefit at the 12- month endpoint (Marks *et al.*, 2010). Interestingly, patients who had a longer blinded follow-up with evaluations at 15 to 18 months did show a significant benefit in favour of the AAV2-NTN treatment, raising the possibility that there may be delayed benefit. However, even though NTN was stably and highly expressed in the striatum, there did not seem to be any retrograde axonal transport to the SN (Marks *et al.*,

2010). As a result, following pre-clinical validation of the surgical approach, Ceregene initiated clinical trials infusing AAV2-NTN in both the putamen and the SN in order to optimize target delivery. AAV2-NTN delivery bilaterally to both anatomical sites proved to be safe in a phase I trial (Bartus *et al.*, 2013b), but unfortunately, the subsequent double-blind trial performed on 52 subjects did not meet the primary endpoint in efficacy (Olanow *et al.*, 2015). Interestingly, patients with disease duration of less than 5 years did show a clinical benefit compared to controls, raising questions of the appropriate disease stage for neurorestorative therapies.

PDGF-BB

Based on the pre-clinical evidence, safety and tolerability of PDGF-BB was investigated in patients with moderate PD in a phase I/IIa first-in-man clinical trial. Twelve patients with moderate PD received either rhPDGF-BB or placebo administered via ICV for 2 weeks and were followed for 3 months thereafter. The study confirmed that PDGF-BB is well tolerated and safe. Importantly, patients receiving the highest dose of growth factor showed a significant improvement in DAT binding, a marker of DA fibers, as measured by PE2I-PET-scan, whereas the signal declined in placebo patients and low dose patients, indicating ongoing neurodegeneration (Paul *et al.*, 2015). This indicates that this treatment may have potential disease-modifying effects for PD patients, keeping in mind that patient numbers were small in this study as it was not designed or powered to measure efficacy. Interestingly, at the end of the 3-month follow-up period, there was an improvement in the Unified Parkinson's Disease Rating scale (UPDRS) part III motor scores in all cohorts, including the placebo-treated patients. It therefore still remains speculative whether the findings related to PDGF-BB in this study reflect a clinical improvement, and further clinical trials addressing efficacy in particular are warranted.

CDNF

CDNF is currently under investigation by Herantis in a first-in-human double-blind, placebo-controlled clinical study in patients with PD. Twenty patients with advanced PD will be recruited at clinical centers in Helsinki, Finland, Stockholm, and Lund, Sweden (www. treatER.com; ClinicalTrials.gov Identifier: NCT03295786). The growth factor is delivered via a CED system by monthly infusion over a treatment period of 6 months followed by an open-labeled extension. First results are likely to be reported by the end of 2020.

4. Caveats of clinical trial design and interpretation of clinical results

A recent systematic review and meta-analysis of the data from all of the GDNF and neurturin clinical trials found no significant effect on motor symptoms, compared to placebo (Hegarty et al., 2017). Although this analysis was limited by the small number of trials that have been conducted to date, it highlights the need for further preclinical work and careful consideration of all aspects of the design of future clinical trials. There is a critical need to understand the reasons for the failures of the double-blind placebo-controlled clinical trials for GDNF and NTN, especially with regards to the very promising pre-clinical data. Generally, it is important to consider the mechanism of the factor applied and whether the delivery method, duration of treatment and dose are sufficient for the drug to reach its target and exert its effect. Are there biological reasons for trial failure, such as neutralizing antibodies? Is the disease too advanced to allow clinically detectable effects of neurorestoration? Does the substance actually reach its target or should it be delivered in a different way? Is the dose sufficient in humans? Dose extrapolations from animal data to humans are complex and not only based on body weight, but also organ size, and species-specific differences in metabolism etc. What should we target: ER stress, mitochondrial dysfunction, protein aggregation, inflammation, vascular leakage, dopamine-synthesising enzymes? Importantly, is the

biological mechanism of the intervention known, is it translatable to human disease and does it affect the study design? What lessons should we draw from previous trials when moving on?

When performing clinical studies, trial design proves to be absolutely crucial. There are a number of caveats that influence the trial outcome and may lead to trial failure and thereby withhold an effective therapy for patients who otherwise might benefit from this treatment. Patient selection and stratification are of utmost importance, as patients with PD have a large variability in clinical symptoms. Several studies have shown upon post hoc analysis that often patients who were younger and had less disease duration or less severe symptoms at baseline had a significant clinical benefit. In particular, exploratory analysis of the two phase II AAV2-NTN clinical trials indicated that subjects with a disease duration < 5 years experienced positive effects on motor symptoms compared to baseline, whereas individuals with a disease duration of >10 years did not show any improvement (Bartus, 2015; Olanow *et al.*, 2015). All three subjects with a disease duration < 5 years who were included in the first AAV2-NTN phase II trial (Marks *et al.*, 2010; Bartus *et al.*, 2013b) showed a very robust response to treatment (Bartus, 2015). Noteworthy, the participants in the open-labeled GDNF trials (Gill *et al.*, 2003; Slevin *et al.*, 2005) had generally milder disease at enrolment than those participating in the phase II study (Lang *et al.*, 2006) (mean off motor scores: 34 (25 to 42) (Gill *et al.*, 2003) and 40 ± 4 (SE) (Slevin *et al.*, 2005) vs 43 (29 to 73) (Lang *et al.*, 2006), respectively).

These exploratory analyses point to the importance of performing neuroprotective studies at a disease stage that allows the intervention to exert an effect. The choice of time point for the intervention along the time line of disease progression is one, if not the major, caveat for studies testing neuroprotective interventions. Most, if not all, trophic factors rely on a remaining number of DA cells and/or fibers, which implies that patients should not be too advanced in their disease in order to enable the factor to have a protective and restorative effect. Ideally, neuroprotective and neurorestorative factors should be applied in the pre-

symptomatic phase, when symptoms are masked by compensatory mechanisms, but cell and fiber degeneration are already ongoing. Advances in biomarker research will hopefully lead to earlier diagnosis and allow implementation of neuroprotective therapies at less advanced stages of disease, when the nigrostriatal pathway is sufficiently intact to respond to the trophic factors. Recently, evidence has been emerging for axonal degeneration as an early stage in nigrostriatal DA loss in PD (Tagliaferro & Burke, 2016; O'Keeffe & Sullivan, 2018). The neuroprotective action of NTFs relies on their retrograde transport from striatal axonal terminals to the cell bodies in the SN. Thus, early dying-back of nigrostriatal axons and consequent impairment of axonal transport may limit the efficacy of exogenously-administered NTFs. In the future, strategies aimed at promoting the re-growth of damaged axons may be used in combination with NTFs, to improve clinical outcomes.

The outcome parameters chosen for a clinical trial define what we measure, and we need to carefully consider when is a reasonable time point to measure these parameters and what differences represent a clinically relevant effect. Most clinical trials investigating NTFs have shown an additional improvement at later time points. Thus, in the initial phase II AAV2-NTN trial, improvement in the primary clinical endpoint was seen at 15–18 months versus the original end of study date at 12 months, suggesting that longer-term assessments might be essential to identify a clinical benefit (Marks *et al.*, 2010). Similar insights have come from case reports (Love *et al.*, 2005; Slevin *et al.*, 2007; Patel *et al.*, 2013) describing sustained and increasing benefit over several years in patients who had received GDNF in the open-labeled trials.

Reasons for the differences between open-label trials often showing substantial benefit and poor results of double-blind placebo-controlled studies are often attributed to placebo effects. A strong placebo effect is a well-described phenomenon in PD trials (Goetz *et al.*, 2008) and is especially evident with complex and invasive interventions. Placebo effects

versus predicted controls need to be taken into consideration when calculating the power for a clinical study so that lack of efficacy reported is not due to underpowered studies. Further challenges in clinical trials include subjective assessment tools and the lack of validated surrogate measures proving target engagement and correlating with clinical improvement. Usually, we are mainly concerned with type I errors in clinical study design, that is when an ineffective intervention appears effective. However, truly effective therapies may be overlooked due to the study design, ultimately leaving the patients without access to a potentially beneficial therapy (type II error). This emphasizes that clinical trials need to be sufficiently powered to detect significant differences between groups, especially since the variability in the PD patient population is high (Hutchinson *et al.*, 2007).

5. Outlook

Based on pre-clinical studies, NTFs offer promising therapy concepts for PD. However, only a few have reached the clinical testing phase, and the outcome of these clinical trials has generally been disappointing. Is it time to give up? Or is it time to rethink? Should we instead inspect trials for clear “proof of principle” evidence and, rather than terminating trials and moving on to the next (equally doomed) trial, try to identify the reason for failure? We are performing research in a time where fast reward is demanded by sponsors, which entails the risk that we may miss a potentially potent treatment by rushing forward. We need to consider that it may be advantageous to carefully select a homogenous patient population, to test neurorestorative therapies much earlier in the disease, to wait long enough to increase the chance of detecting a meaningful clinical improvement, and to carefully select outcome measures. We owe the PD patient population our continuous effort to prevent disease progression.

6. Author contributions

AS and GP wrote the article. AS wrote the majority of the preclinical part and GP the part of PDGF-BB and the clinical part.

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8. Conflict of interest

No conflict of interest to declare.

9. References

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Growth factor	delivery	Trial type	Patient nr enrolled	reference
GDNF	icv	Phase I/II	50	(Nutt *et al.*, 2003)
	putamen	Phase I	5	(Gill *et al.*, 2003; Patel *et al.*, 2005)
	putamen	Phase I	10	(Slevin *et al.*, 2005; Slevin *et al.*, 2007)
	putamen	Phase II	34	(Lang *et al.*, 2006)
	putamen (CED)	Phase II	41	www.parkinsons.uk
AAV2-GDNF	putamen (CED)	Phase I	25	NCT01621581
AAV2-NTN	putamen	Phase I	12	(Marks *et al.*, 2008)
	putamen	Phase II	58	(Marks *et al.*, 2010)
	putamen, SNc	Phase I	6	(Bartus *et al.*, 2013)
	putamen, SNc	Phase II	52	(Olanow *et al.*, 2015)
PDGF-BB	icv	Phase I	12	(Paul *et al.*, 2015)
CDNF	putamen (CED)	Phase I	18	NCT03295786

Table 1. Previous and current clinical trials investigating trophic factors in PD.